

Direct Screening Methods for Rapid Identification of KPC-Producing *Klebsiella Pneumoniae* and *Escherichia coli*

Thesis

*Submitted for Partial Fulfillment of Master Degree
in Clinical and Chemical Pathology*

Presented by

Walaa Ahmed Ahmed Ahmed Barakat
(M.B., B.Ch.) Ain Shams University

Under Supervision of

Professor/ Névine Nabil Kassem

*Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University*

Professor/ Omnia Abou El-Makarem Shaker

*Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University*

Doctor/ Sherin Ahmed El-Masry

*Lecturer of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University*

**Faculty of Medicine
Ain Shams University**

2012

**طريقة الفحص المباشر للتعرف على كلبسيلا الإلتهاب الرئوي
والإشريشية القولونية المنتجين لكاربايناميز كلبسيلا الإلتهاب
الرئوي في العينات الإكلينيكية**

رسالة

توطئة للحصول على درجة الماجستير في الباثولوجيا الإكلينيكية والكيميائية

مقدمة من

الطبيبة/ ولاء أحمد أحمد أحمد بركات

بكالوريوس الطب والجراحة العامة

كلية الطب جامعة عين شمس

تحت إشراف

أ.د/ نيفين نبيل قاسم

أستاذ الباثولوجيا الإكلينيكية والكيميائية

كلية الطب - جامعة عين شمس

أ.د/ أمنية أبو المكارم شاكر

أستاذ الباثولوجيا الإكلينيكية والكيميائية

كلية الطب - جامعة عين شمس

د/ شيرين أحمد المصري

مدرس الباثولوجيا الإكلينيكية والكيميائية

List of Contents

Title	Page
♦ Introduction	1
♦ Aim of the Work	4
♦ Review of Literature:	
▪ Chapter 1:	
○ Mechanisms of Antibiotic Resistance in Klebsiella Pneumoniae and Escherichia Coli.....	5
▪ Chapter 2:	
○ Carbapenems and Carbapenemases.....	26
▪ Chapter 3:	
○ Klebsiella Pneumoniae Carbapenemases	38
▪ Chapter 4:	
○ Laboratory Detection of KPC Producing Organisms	48
▪ Chapter 5:	
○ Treatment	80
▪ Chapter 6:	
○ Prevention and Control	87
♦ Materials and Methods	90
♦ Results	123
♦ Discussion	136
♦ Conclusion and Recommendations	149
♦ Summary	153
♦ References	157
♦ Arabic Summary	--

List of Tables

Table No.	Title	Page
1	Selected pathogens, resistance phenotypes and underlying mechanisms.	18
2	Comparison of Beta-Lactamase Classification Systems.	25
3	Groups and examples of β -lactam antimicrobial agents	27
4	Carbapenemases (classification, activity and producing organisms).	35
5	CLSI recommended screening guidelines for carbapenemase.	57
6	Carbapenem breakpoints.	58
7	Primers sequences.	96
8	The biochemical reactions of <i>E.coli</i> and <i>k.pneumoniae</i> strains.	108
9	Zone diameter interpretive standard for the tested antibiotics.	110
10	E-test MIC values for imipenem and meropenem	113
11	Components of PCR tube	118
12	Distribution of <i>k. pneumoniae</i> and <i>E.coli</i> isolates according to the type of specimen.	123
13	Distribution of <i>k. pneumoniae</i> and <i>E.coli</i> isolates according to the department.	124
14	Antibiotic resistance pattern of isolated <i>k. pneumoniae</i> and <i>E.coli</i> to different antibiotics by disc diffusion method.	125

List of Tables (Cont.)

Table No.	Title	Page
15	Results of carbapenem resistance among 100 isolates of <i>K. pneumoniae</i> and <i>E.coli</i> by 2 screening methods and disc diffusion.	127
16	Distribution of 15 resistant isolates by E-test according to the department.	128
17	Distribution of 15 resistant isolates by E-test according to the type of specimen.	128
18	Comparison between method 1 and confirmatory test (E- Test).	130
19	Diagnostic characteristics of method 1 to diagnose resistance considering confirmatory test(E-Test) as a reference method.	131
20	Comparison between method 2 and confirmatory test (E-Test).	132
21	Diagnostic characteristics of method 2 to diagnose resistance considering confirmatory test(E-Test) as a reference method.	133

List of Figures

Figure No.	Title	Page
1	Efflux systems reverse the diffusion of antibiotics across the OM.	8
2	Outer wall of Gram-positive and Gram-negative species and detail of porin channels of Gram-negative bacteria.	20
3	β -Lactamase found in bacteria and their classification and synthesis, whether chromosomally or plasmid mediated.	22
4	Klebsiella pneumoniae isolate tested with Imipenem E-test strip (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar.	52
5	Susceptibility testing: E-test methodology	56
6	CHROMagar KPC.	61
7	Potential of carbapenems by APB in K. pneumoniae producing KPC-2.	65
8	Results of DDST with ETP.	67
9	The modified Hodge Test.	69
10	Results obtained with the modified Hodge test (MHT), the boronic acid-based MHT (BA-MHT), and the OXA-based MHT.	72
11	5ml tryptone soya broth contain 10 μ g imipenem disc.	100
12	MacConkey agar plate showing ertapenem resistant k.pneumoniae.	102

List of Figures (Cont.)

Figure No.	Title	Page
13	β -Lactamase found in bacteria and their classification and synthesis, whether chromosomally or plasmid mediated	108
14	Klebsiella pneumoniae isolate tested with Imipenem E-test strip (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar.	109
15	Meropenem E-test strip.	112
16	Agarose gel electrophoresis	121
17	Antibiotic resistance pattern of isolated k. pneumoniae and E.coli to different antibiotics by disc diffusion	126
18	Diagnostic characteristics of methods 1 and 2 to diagnose resistance considering confirmatory test (E-Test) as a reference method in the whole sample.	134
19	Diagnostic characteristics of methods 1 and 2 to diagnose resistance considering confirmatory test (E-Test) as a reference method in K.pneumoniae samples.	135
20	Diagnostic characteristics of methods 1 and 2 to diagnose resistance considering confirmatory test (E-Test) as a reference method in E.coli.	135

List of Abbreviations

ABC	Adenosine triphosphate (ATP)-binding cassette
AcrAB	Acriflavine resistance protein A and B
AK	Amikacin
ALG	Alginate
AMC	Amoxicillin- clavulanic acid
AmpC	Ambler Class C
APB	aminophenyl-boronic acid
BA-CD	Boronic acid combined disc test
bla	Beta -lactamase
BMD	Broth microdilution
CAZ	Ceftazidime
CDC	Center for Disease Control and Prevention
CFP	Cefoperazone
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standards Institute
CM	Cytoplasmic membrane
CP	Carbapenemase-producing
CPD	Cefpodoxime
CRO	Ceftriaxone
CTX	Cefotaxime
CTX-Ms	Cefotaximase
ddNTP	dideoxynucleotide triphosphate
DDST	Double disc synergy test
<i>E. coli</i>	<i>Escherichia coli</i>
E-test	Epsilometer test
EDTA	Ethylene-diamine-tetra-acetic acid
ESBLs	Extended-spectrum β lactamases
EU	European Union
F	Nitrofurantoin

FOX	Cefoxitin
GES	Guiana extended spectrum beta- lactamase
GIM	German imipenemase
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
IBC	Integron borne cephalosporinase
ICU	Intensive care unit
IEF	Isoelectric focusing
IM	inner membrane
IMI	Imipenem hydrolyzing β - lactamase
IMP	Inner membrane proteins
IND	indologenes
IPM	Imipenem
K.	<i>Klebsiella</i>
KPC	<i>Klebsiella pneumoniae</i> carbapenemase
LPS	Lipopolysaccharide
MATE	Multidrug and toxic compound extrusion
MBLs	Metallo- β -lactamases
MB-PCR	molecular beacons-polymerase chain reaction
MDR	Multi drug resistant
MEM	Meropenem
MFP	membrane fusion protein
MFS	Major facilitator superfamily
MHT	Modified Hodge Test
MIC	Minimum inhibitory concentration
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
NDM	New Delhi metallo- β -lactamase
NMC	Non metalloenzyme carbapenemase
OM	Outer membrane
OMP	Outer membrane proteins
OmpC	Outer membrane protein C

OmpF	Outer membrane protein F
Omp K35	Osmoporins of <i>klebsiella pneumoniae</i>
OmpK36	Osmoporins of <i>klebsiella pneumoniae</i>
OXA	Oxacillin
OXA-MHT	Oxacillin –Modified Hodge Test
P.	<i>Pseudomonas</i>
PBP_s	Penicillin Binding Proteins
PC1	penicillinases 1
PCR	Polymerase Chain Reaction
PFGE	Pulsed- Field Gel Electrophoresis
PG	Peptidoglycan
pIs	Isoelectric points
Qnr	quinolones resistance
RND	Resistance-nodulation-cell-division
S.	<i>Streptococcus</i>
<i>S. marcescens</i>	<i>Serratia marcescens</i>
SHV	sulfhydryl variable
SIM	Seoul imipenemase
SME	<i>Serratia marcescens</i> enzyme
SMR	Small multidrug resistance
SPM	San Paulo metallo-β-lactamase
Spp.	Species
SXT	Trimethoprim-Sulfamethoxazole
TEM	Temoneira (name after the patient providing the first sample)
TZP	Tazobactam
US	United States
UTI	Urinary tract infection
VIM	Verona integron-encoded metallo-β-lactamase
Zn	Zinc



Acknowledgment

First of all, thanks to **Allah** for helping and guiding me in accomplishing this work and for everything else I have.

Words are not sufficient to express my sincerest appreciation and my deepest gratitude to **Professor/ Névine Nabil Kassem**, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her continuous encouragement, and her precious remarks which guide me to present this work in its proper way, it was indeed an honor to have been supervised by her.

I would like to thank **Professor/ Omnia Abou El-Makarem Shaker**, Professor of Clinical and Chemical Pathology, for her guidance, meticulous revision and suggestions which were of great value to me.

I would like to deeply thank **Doctor/ Sherin Ahmed El-Masry** Lecturer of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her continuous guidance and suggestions which were of great value to me, and her extreme support.

An endless thanks for my father, my mother and all my family special thanks to Wael Barakat and Amal Barakat, for their support without it, I would never completed this work.

✍ **Walaa Ahmed Barakat**



قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا
إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

سورة البقرة الآية (٢٢) 4

**طريقة الفحص المباشر للتعرف على كلبسيلا الإلتهاب الرئوي
والإشريشية القولونية المنتجين لكاربايناميز كلبسيلا الإلتهاب
الرئوي في العينات الإكلينيكية**

رسالة

توطئة للحصول على درجة الماجستير في الباثولوجيا الإكلينيكية والكيميائية

مقدمة من

الطبيبة/ ولاء أحمد أحمد أحمد بركات
بكالوريوس الطب والجراحة العامة
كلية الطب جامعة عين شمس

تحت إشراف

أ.د/ نيفين نبيل قاسم

أستاذ الباثولوجيا الإكلينيكية والكيميائية
كلية الطب - جامعة عين شمس

أ.د/ أمنية أبو المكارم شاكر

أستاذ الباثولوجيا الإكلينيكية والكيميائية
كلية الطب - جامعة عين شمس

د/ شيرين أحمد المصري

مدرس الباثولوجيا الإكلينيكية والكيميائية

Introduction

The growing increase in the rates of antibiotic resistance is a major cause for concern in both nonfermenting bacilli and isolates of the Enterobacteriaceae family. β -Lactams have been the mainstay of treatment for serious infections, and the most active of these are the carbapenems, which are advocated for use for the treatment of infections caused by extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae*, particularly *Escherichia coli* (*E.coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) (**Kaul and Chhina, 2010**).

Carbapenem resistance among Enterobacteriaceae, in particular *K. pneumoniae* and *E.coli*, is an emerging problem worldwide. Several resistance mechanisms have been reported to circumvent the efficacy of carbapenems, and carbapenemases (carbapenem- hydrolyzing β -lactamases) are the most prominent enzymes that neutralize carbapenems. Class A carbapenemases, which include bla_{KPC}, NMC, SME-1 to -3, IMI-1, and GES, have been characterized in several genera of the family Entero-bacteriaceae (**Wang et al., 2012**).

Klebsiella pneumoniae carbapenemase (KPC) is a molecular class A serine β -lactamase belonging to functional group 2f (**Fontana et al., 2010**). The KPC β -lactamase occurs most commonly in *K. pneumoniae*, but it has also been reported

sporadically in other species of Enterobacteriaceae (*Klebsiella oxytoca*, *Enterobacter* spp., *E.coli*, *Salmonella* spp., *Citrobacter freundii*, and *Serratia* spp.) and *Pseudomonas aeruginosa* (*P.aeruginosa*). The KPC enzyme confers resistance to all β -lactam agents including penicillins, cephalosporins, monobactams, and carbapenems (**Francis et al., 2012**).

The patient groups most likely to acquire KPC-producing bacteria include the patients at risk for infections caused by multidrug resistant organisms: patients with invasive devices, prolonged hospital stays (especially in an ICU), and heavy antibiotic exposure and those who are immunocompromised (**Arnold et al., 2012**).

The *bla_{KPC}* gene is plasmid mediated and is carried in a Tn3-based transposon, Tn4401; the potential ease of mobility of this resistance mechanism is a major concern (**Kitchel et al., 2009**). This plasmid also, often contains genes that code for resistance to non β -lactam agents such as aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole (**Wang et al., 2012**). Therefore, it is important to isolate infected patients and take contact precautions because of the potential for nosocomial transmission (**Toye et al., 2009**). Also, controlled antibiotic usage must be complemented by the utilization of rapid and sensitive *bla_{KPC}* diagnostic assays (**Hindiyeh et al., 2008**).